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Integrative Functional Genomics and Proteomics to Uncover Mechanisms of Resistance to Chemotherapy in Ovarian Cancer

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TABLE OF CONTENTS

INTRODUCTION: Ovarian cancer affects approximately 22,000 women and kills approximately 16,000 women each year in the United States. Resistance to chemotherapy is the major contributing factor in ovarian cancer-related death. The primary objective of this study is to gain mechanistic insights into the development of chemotherapy resistance in ovarian cancer. We expect that advances in this study will contribute to our understanding of chemotherapy resistance in ovarian cancer and will facilitate the development of more effective therapies to overcome the problems of chemotherapy resistance.

BODY: In the approved SOW for Year 1, we expected to accomplish the following three main tasks:

1. Generate expression data from ovarian tumors
2. Generate expression data from cancer cell lines
3. Analysis of expression data from ovarian tumors and cell lines

In order to accomplish the first task, we successfully obtained the approval from the institutional review board for the use of human tumor specimens for gene expression analysis. We also successfully identified biospecimens to be used in the study (Table 1). Patients with differential response to chemotherapy were classified as resistant, responsive and sensitive to platinum-based therapy based on the duration of treatment-free interval (TFI). In the clinical setting, patients with TFI<=6 months are considered resistant to chemotherapy.[1] Therefore, our TFI values follow clinical definition of resistance. To identify mechanisms associated with chemotherapy resistance, we plan to compare two groups of patients with extreme differential response to chemotherapy: those who are resistant to chemotherapy and those who are extremely sensitive to chemotherapy (TFI>18 months). In this study design, we excluded the intermediate group that are responsive to chemotherapy but do not have extreme sensitivity. The rationale for excluding the intermediate group is that multiple mechanisms could account for the intermediate response phenotype and that inclusion of this group may confound the analysis to identify differentially expressed genes and altered biological pathways associated with differential response to chemotherapy. Although we had previously planned to finish the expression analysis of these tumors within 6 month from the start of funding, we were not able to accomplish the task on time due to re-evaluation and assessment of competing technologies to obtain the most cost-effective expression data from these tumors. While preparing for the generation of expression data from tumor samples, we evaluated whole genome DASL,[2] as originally proposed, as well as transcriptomic sequencing. Although the transcriptomic sequencing approach is slightly more expansive than the whole genome DASL approach, the former approach produces high-quality expression data that can be used to determine not only expression of genes but also the differences in alternative splice forms and novel transcripts that cannot be obtained from the whole genome DASL. With substantial cost reduction in sequencing technology as the technology becomes more mature, more widely adopted, and more affordable, we expect the expression analysis by sequencing will be as affordable as the whole genome DASL approach. Therefore, we delayed initial analysis of gene expression from the tumors. We expect to complete this task in the first quarter of Year 2

funding calendar, and we expect to use the budget carried over from last year to complete the gene expression analysis.

Classification	Resistant	Responsive	Sensitive
Definition	(TFI<=6m)	(6m<TFI<18m)	(TFI>18m)
Total cases	380 (36%)	232 (22%)	438 (42%)
Endometrioid	37	31	46
Serous	189	123	227
Others	154	78	165

Table 1. Review of biospecimens for the study. We will use 30 serous and 30 endometrioid tumors each belonging to the Resistant and Sensitive groups in the next phase of research studies.

In order to accomplish the second task, we had generated half-maximal inhibitory concentration (IC_{50}) for cisplatin and paclitaxel as proposed in the approved SOW. We proposed to generate IC_{50} for 23 ovarian cancer cell lines, and we have accomplished this task. The results, shown in Figure 1 and 2, indicate variable levels of IC_{50} for cisplatin and paclitaxel in select ovarian cancer cell lines.

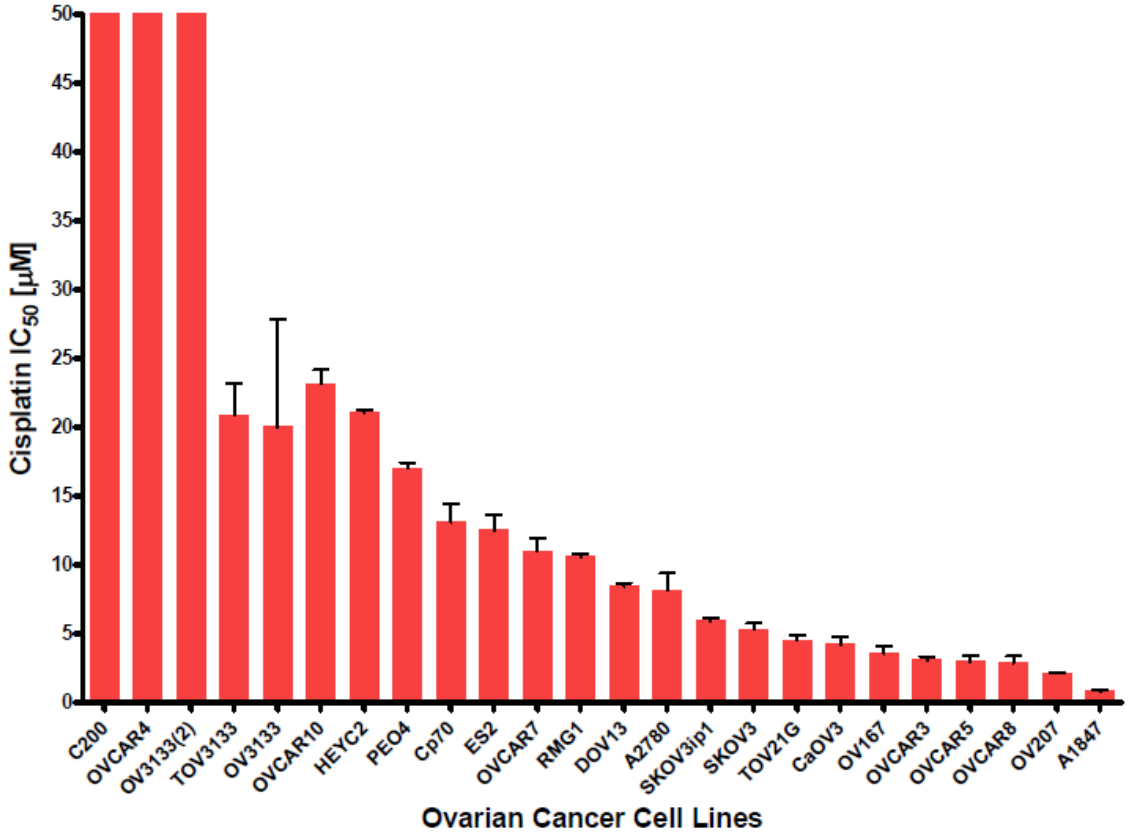

Figure 1. Cytotoxicity profile of ovarian cancer cell lines treated with cisplatin. Ovarian cancer cells were grown in a unified medium (MCDB-105/M199 with 5% FBS) and treated with varying concentrations of cisplatin. Following 2-day of cisplatin treatment, MTT assay was performed to determine cell viability. IC_{50} was calculated using

sigmoidal dose-response curve analysis from three independent experiments. C200, OVCAR4, and OV3133(2) displayed high IC_{50} values which were outside the scale of graph presented in Figure 1.

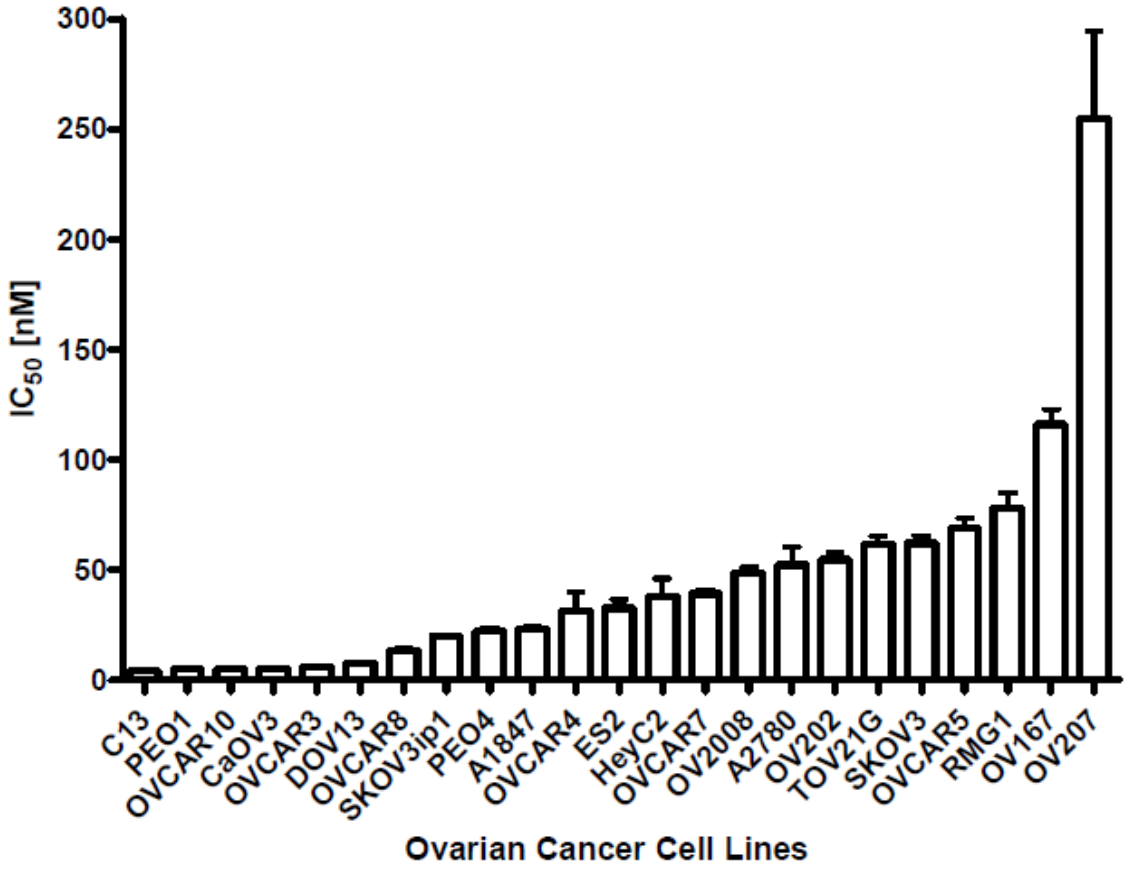

Figure 2. Cytotoxicity profile of ovarian cancer cell lines treated with paclitaxel. Ovarian cancer cells were grown in a unified medium (MCDB-105/M199 with 5% FBS) and treated with varying concentrations of paclitaxel. Following 3-day of paclitaxel treatment, MTT assay was performed to determine cell viability. IC_{50} was calculated using sigmoidal dose-response curve analysis from three independent experiments.

In order to accomplish the third task, we had collected RNA samples from 23 ovarian cancer cell lines. We are in the process of generating expression data from these RNA samples. As a pilot study, we performed mRNA-sequencing of 24 tumor samples to assess the data quality and output from the HiSeq-2000 sequencer. One sample each was run on each lane of the flow cell on HiSeq2000. The resulting sequences were analyzed with TopHat software,[3] and the resulting aligned BAM files were analyzed by SAMStat.[4] The results shown in Figure 3, indicate that each lane from the flow cell produced approximately 200 million reads that were mapped to the genome. Approximately 88% of all reads are mapped to the genome with the quality score of >=30 (Figure 3).

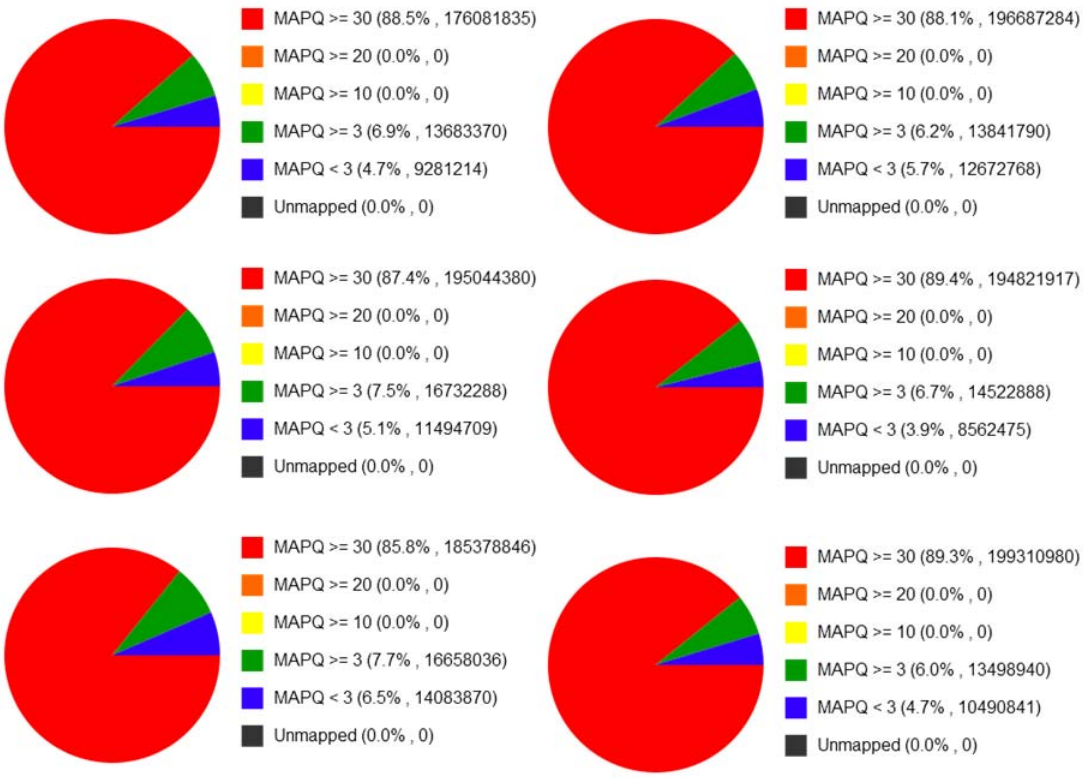

Figure 3. Analysis of mapped reads from mRNAseq data from the representative 6 tumor samples. As many as 199 million high quality mapped reads (MAPQ>=30) and as few as 176 million high quality mapped reads are observed from these data. Based on the comparison of array-based MAQC data and mRNAseq data, it is estimated that 15 millions of reads are needed to discover 80% of total expressed transcripts.[5] Based on these data and our own data, we expect at least 17 million reads from a 10-plexed samples from one lane of HiSeq2000 flow cell. Sequencing cost of 10-plexed samples will result in approximately $100 per sample. The library cost for each sample is around $66 per library. Therefore, the cost analysis indicates that total sequencing cost, including library, will be around $166 per sample. This level of transcript coverage from mRNAseq data at reasonable sequencing cost is not possible with array-based approaches.

We therefore request an approval for a minor change in the Statement of Work from the Army Grants Officer's Representative to perform mRNAseq of the samples rather than the array based approach that we had originally proposed.

KEY RESEARCH ACCOMPLISHMENTS:

- IRB approval obtained
- Tumor samples selected and method to generate gene expression from these tumors evaluated
- IC_{50} for ovarian cancer cell lines established
- RNA from cell lines collected for gene expression analysis

REPORTABLE OUTCOMES: The overview of DoD-funded research program was presented at the first annual DoD OCRP Academy meeting at Baltimore in 2010. Data generated from the funded study was also used to support two grant applications submitted to the Minnesota Ovarian Cancer Alliance and Marsha Rivkin Center Pilot Study Award. This funding from the DoD OCRP Academy has resulted in the employment of 1 FTE as a research fellow. The funding support has also allowed me to develop tissue resources, primary cultures, cell lines, and external collaborations to forge studies in chemotherapy resistance. One manuscript stemming from these studies is in preparation.

CONCLUSION: During the first year of funding, I have met regulatory requirements for research, established necessary resources, hired personnel, and evaluated competing technologies to obtain the most cost-effective and comprehensive data on gene expression. In particular, due to the reduction in sequencing cost associated with Illumina Genome Analyzer sequencing technology, we were able to evaluate the feasibility and cost-effective approach in gene expression analysis using mRNA sequencing by Illumina HiSeq 2000. We will therefore use this approach to develop gene expression data, alternative splicing data, and variant and novel transcript data associated with chemotherapy resistance. This approach will be far superior to originally proposed approach of using whole genome DASL, in which approximately 20,000 known transcripts are to be analyzed. In contrast, with the sequencing approach, we will be able to generate quantitative gene expression analysis, alternative splicing events, novel transcripts, and variant transcripts associated with chemotherapy response. This comprehensive dataset will allow us to meet and exceed the goals set out in the study. This dataset will help us identify critical genes, alterations, and biological pathways associated with chemotherapy resistance, and we expect this knowledge will translate into clinical applications in the form of targeted therapies to overcome chemotherapy resistance.

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